CLEAN VERSION OF REWRITTEN, ADDED, AND/OR CANCELLED CLAIMS PURSUANT TO 37 C.F.R. §1.121 (c)(1)(i)

IN THE CLAIMS:

Cancel Claims 13, 27, 30, 31, 33, and 34.

Replace Claims 1, 15, 35, and 36, with the following claims that have corresponding numbers, and add new Claims 37-50 as follows:

1. (Four times amended) A method of producing a modification in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos;

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest and embryonic cells having a modified gene of interest; and

c) isolating said embryonic cells having a modified gene of interest.

15. (Four times amended) A method of producing an allelic series of modifications in a gene of interest contained in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos;

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest, embryonic cells having a first modification in said gene of interest, and embryonic cells having a second modification in said gene of interest; and

c) isolating said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic cells.

35. (Twice amended) A method of producing a modification in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos;

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest and embryonic cells having a modified gene of interest;

c) isolating said embryonic cells having a modified gene of interest; and

d) placing at least one of said embryonic cells having a modified gene of interest into an environment under conditions so as to generate a non-human animal comprising said modified gene of interest.

36. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos;

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest, embryonic cells having a first modification in said gene of interest, and embryonic cells having a second modification in said gene of interest;

c) isolating said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic cells; and

d) placing at least one embryonic cell selected from the group consisting of said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest into an environment under conditions so as to generate a non-human animal comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

37. (New) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) a chemical agent capable of producing at least one modification in said gene of interest;

b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and (ii) a mixture of embryonic stem

cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

38. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 70% of the genes in said mouse embryonic stem cells is produced.

39. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 85% of the genes in said mouse embryonic stem cells is produced.

40. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 95% of the genes in said mouse embryonic stem cells is produced.

41. (New) The method of Claim 37, wherein the number of said isolated mouse embryonic stem cells in said *in vitro* culture consists of from 200 to 600 embryonic stem cells, and said chemical agent is *N*-ethyl-*N*-nitrosourea.

42. (New) The method of Claim 37, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

43. (New) The method of Claim 37, wherein said gene of interest is associated with a disease.

44. (New) The method of Claim 43, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.

45. (New) The method of Claim 37, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.

46. (New) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) *N*-ethyl-*N*-nitrosourea;

b) treating said mouse embryonic stem cells with said *N*-ethyl-*N*-nitrosourea to produce treated mouse embryonic stem cells comprising a mixture of embryonic stem cells, said mixture comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest, wherein the treatment is under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9,000; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

47. (New) The method of Claim 46, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.

48. (New) The method of Claim 46, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

49. (New) The method of Claim 46, wherein said gene of interest is associated with a disease.

50. (New) The method of Claim 49, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.

APPENDIX II

CLEAN VERSION OF THE ENTIRE SET OF PENDING CLAIMS AS AMENDED IN THIS COMMUNICATION

The following is a list of the pending claims as they would appear following entry of the Examiner's amendment which appears in the Office Action mailed on March 15, 2001, and entry of this amendment.

1. (Four times amended) A method of producing a modification in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos;

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest and embryonic cells having a modified gene of interest; and

c) isolating said embryonic cells having a modified gene of interest.

2. (Once amended) The method of Claim 1, further comprising step d) comparing the nucleotide sequence of said gene of interest in said embryonic cells having a modified gene of interest with the nucleotide sequence of said gene of interest in said embryonic cells having an unmodified gene of interest.

3. (Three times amended) The method of Claim 1, further comprising step d) placing at least one of said embryonic cells having a modified gene of interest into an environment under conditions so as to generate a non-human animal comprising said modified gene of interest.

4. The method of Claim 2, further comprising prior to step d) amplifying said modified gene of interest to produce an amplified modified gene of interest.

5. The method of Claim 4, further comprising prior to step d) sequencing said amplified modified gene of interest.

6. The method of Claim 1, wherein said modification is selected from the group consisting of mutation, mismatch, and strand break.

7. The method of Claim 6, wherein said mutation is selected from the group consisting of deletion, insertion and substitution.

8. The method of Claim 6, wherein said strand break is selected from the group consisting of single-strand break and double-strand break.

10. (Once amended) The method of Claim 1, wherein said target cells are isolated from a mammal.

11. The method of Claim 10, wherein said mammal is a mouse.

12. (Once amended) The method of Claim 1, wherein said target cells are isolated from a zebrafish.

14. (Twice amended) The method of Claim 1, wherein said chemical agent is selected from the group consisting of *N*-ethyl-*N*-nitrosurea, methylnitrosourea, procarbazine hydrochloride, triethylene melamine, acrylamide monomer, chlorambucil, melphalan, cyclophosphamide, diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, 6-mercaptopurine, mitomycin-C, procarbazine, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, $^{3}H_2O$, and urethane.

15. (Four times amended) A method of producing an allelic series of modifications in a gene of interest contained in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos;

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest, embryonic cells having a first modification in said gene of interest, and embryonic cells having a second modification in said gene of interest; and

c) isolating said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic cells.

16. (Twice amended) The method of Claim 15, further comprising step d) comparing the nucleotide sequence of said gene of interest in said embryonic cells having an unmodified gene of interest with the nucleotide sequence of said gene of interest in embryonic cells selected from the group consisting of said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest.

17. (Three times amended) The method of Claim 15, further comprising step d) placing at least one embryonic cell selected from the group consisting of said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest into an environment under conditions so as to generate a non-human animal comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

18. (Once amended) The method of Claim 16, further comprising prior to step d) amplifying said gene of interest selected from the group consisting of said gene of interest having said first modification and said gene of interest having said second modification to produce an amplified modified gene of interest selected from the group consisting of an amplified gene of interest having said first modification and amplified gene of interest having said second modification.

19. The method of Claim 18, further comprising prior to step d) sequencing said amplified modified gene of interest.

20. The method of Claim 15, wherein said first modification and said second modification are selected from the group consisting of mutation, mismatch, and strand break.

21. The method of Claim 20, wherein said mutation is selected from the group consisting of deletion, insertion and substitution.

22. The method of Claim 20, wherein said strand break is selected from the group consisting of single-strand break and double-strand break.

24. (Once amended) The method of Claim 15, wherein said target cells are isolated from a mammal.

25. The method of Claim 24, wherein said mammal is a mouse.

26. (Once amended) The method of Claim 15, wherein said target cells are isolated from a zebrafish.

28. (Twice amended) The method of Claim 15, wherein said chemical agent is selected from the group consisting of *N*-ethyl-*N*-nitrosurea, methylnitrosourea, procarbazine hydrochloride, triethylene melamine, acrylamide monomer, chlorambucil, melphalan, cyclophosphamide, diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, 6-

mercaptopurine, mitomycin-C, procarbazine, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, $^{3}H_2O$, and urethane.

35. (Twice amended) A method of producing a modification in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos;

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified gene of interest and embryonic cells having a modified gene of interest;

c) isolating said embryonic cells having a modified gene of interest; and

d) placing at least one of said embryonic cells having a modified gene of interest into an environment under conditions so as to generate a non-human animal comprising said modified gene of interest.

36. (Twice amended) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture of target cells comprising isolated embryonic cells comprising a gene of interest, said embryonic cells selected from the group consisting of fertilized egg cells and cells of 2-cell embryos;

ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;

b) treating said embryonic cells with said chemical agent under conditions such that a mixture of embryonic cells comprising said gene of interest is produced, said mixture of embryonic cells comprising embryonic cells having an unmodified

gene of interest, embryonic cells having a first modification in said gene of interest, and embryonic cells having a second modification in said gene of interest;

c) isolating said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic cells; and

d) placing at least one embryonic cell selected from the group consisting of said embryonic cells having a first modification in said gene of interest and said embryonic cells having a second modification in said gene of interest into an environment under conditions so as to generate a non-human animal comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

37. (New) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) a chemical agent capable of producing at least one modification in said gene of interest;

b) treating said mouse embryonic stem cells with said chemical agent under conditions such that (i) at least one modification in substantially every gene in said mouse embryonic stem cells is produced, and (ii) a mixture of embryonic stem cells comprising said gene of interest is produced, said mixture of embryonic stem cells comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

38. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 70% of the genes in said mouse embryonic stem cells is produced.

39. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 85% of the genes in said mouse embryonic stem cells is produced.

40. (New) The method of Claim 37, wherein said treating is under conditions such that at least one modification in at least 95% of the genes in said mouse embryonic stem cells is produced.

41. (New) The method of Claim 37, wherein the number of said isolated mouse embryonic stem cells in said *in vitro* culture consists of from 200 to 600 embryonic stem cells, and said chemical agent is *N*-ethyl-*N*-nitrosourea.

42. (New) The method of Claim 37, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

43. (New) The method of Claim 37, wherein said gene of interest is associated with a disease.

44. (New) The method of Claim 43, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.

45. (New) The method of Claim 37, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.

46. (New) A method of producing an allelic series of modifications in a gene of interest in a cell, comprising:

a) providing:

i) an *in vitro* culture comprising isolated mouse embryonic stem cells comprising a gene of interest;

ii) *N*-ethyl-*N*-nitrosourea;

b) treating said mouse embryonic stem cells with said *N*-ethyl-*N*-nitrosourea to produce treated mouse embryonic stem cells comprising a mixture of embryonic stem cells, said mixture comprising embryonic stem cells having a first modification in said gene of interest, and embryonic stem cells having a second modification in said gene of interest, wherein the treatment is under conditions such that the frequency of mutation in any one gene in said treated mouse embryonic stem cells is from 1/600 to 1/9,000; and

c) isolating said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated embryonic stem cells.

47. (New) The method of Claim 46, further comprising step d) detecting at least one of said first and second modification in said gene of interest using fluorescent chemical cleavage of mismatch.

48. (New) The method of Claim 46, further comprising step d) placing at least one embryonic stem cell selected from the group consisting of said embryonic stem cells having a first modification in said gene of interest and said embryonic stem cells having a second modification in said gene of interest into an environment under conditions so as to generate a

mouse comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

49. (New) The method of Claim 46, wherein said gene of interest is associated with a disease.

50. (New) The method of Claim 49, wherein said gene of interest is selected from the group consisting of the p53 gene, BRCA1 gene, PKD1 gene, PKD2 gene, and PKD3 gene.